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Microbial Surfaces in Relation to Pathogenicity

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INTRODUCTION

Pathogenicity is the capacity of microorganisms to produce disease in animal or plant hosts. It is a character possessed by relatively few microbial species and by them to varying degrees. Whether, in a particular instance, disease occurs or is prevented depends on the outcome of interactions between the microbe and the host. Some of these interactions can occur solely between extracellular products such as toxins and antitoxins, but the majority involve surfaces: either those of microbes, as in resistance to humoral defense, or those of host cells, as in damage by toxins, or those of both, as in phagocytosis. Knowledge of the nature of microbial and host surfaces and their role in host-parasite interactions is therefore crucial to a proper understanding of microbial pathogenicity. A book is needed to cover the subject comprehensively, but I hope that some benefit may be derived from a shorter review that deals only with microbes pathogenic for animals and concentrates on the surfaces of microbes rather than those of host cells. Omission of the latter does not detract from the importance of the host in the overall picture. Quite the reverse, since there is neither space available in the article nor has the reviewer sufficient expertise for an adequate treatment of the "other half" of the subject. Indeed, the microbial aspects are so numerous that the review must aim at correlating studies in similar areas and only rarely at describing the details of particular investigations. The plan is as follows.

The facets of pathogenicity that might involve microbial surfaces are defined, and its multifactorial nature is emphasized. Then, since pathogenicity is only manifested in vivo, the possible influences of the environment in vivo on the composition of microbial surfaces are discussed. After this, each facet of pathogenicity is taken in turn. For each, the general principles are outlined first, avoiding detailed discussion of the subject that can be found elsewhere (161, 166, 171). Then, examples are described to show how surface components of bacteria, viruses, fungi, and protozoa might contribute in vivo to the particular facet of pathogenicity under discussion. In this manner, gaps in our knowledge for one particular type of microorganism will be highlighted against more complete information for another type, and possible ways to fill the gaps might become apparent. In most, but not all, cases, studies of bacterial pathogenicity provide the templates for future work. This review does not deal with the general composition of microbial surfaces but only with the questions of whether surface components of pathogens determine disease production in vivo and, if so, their nature. The reader will soon become aware that, in many cases, surface components of microbes are involved in pathogenicity, but only rarely do we know their precise nature. Of the nearly synonymous terms pathogenicity and virulence, the former is used with respect to differences between microbial species and the latter for differences between strains within a species or the same strain grown under different conditions.

FACETS OF PATHOGENICITY THAT CAN BE INFLUENCED BY MICROBIAL SURFACES

To cause disease a pathogen must (i) enter the host, (ii) multiply in host tissues, (iii) resist or not stimulate host defenses, and (iv) damage the host. The mechanisms whereby these essential steps in disease production are accomplished, together with those responsible for host and tissue specificity of infection, form the bulk of knowledge on microbial pathogenicity (171 [p. 1]).The microbial products responsible for these processes are the determinants of pathogenicity (or virulence), and many of them are surface components. Since pathogens must accomplish all four processes to produce disease, and each process is complex, several determinants are usually involved in the overall effect. Lack of one member of the full complement may result in considerable attenuation of the strain concerned. This multifactorial nature of virulence means that a surface component essential to virulence may also be present on a strain that is attenuated through lack of some other member of the full complement (171 [p. 1]). For example, the capsular poly-D-glutamic acid of *Bacillus anthracis* and the surface K88 antigen of *Escherichia coli* are present on attenuated strains that do not possess the toxic factors necessary for virulence (171 [p. 1]).

Viruses need special mention in relation to the different facets of pathogenicity described above. They are different from other microorganisms in that they have unique methods of replication within host cells (58). Sometimes this aspect of pathogenicity – multiplication in vivo – is emphasized at the expense of the others. Virus surface components are certainly involved in replication but, in judging their overall effect, we should remember that virus pathogenicity, like that of other microorganisms, is not determined solely by replication. Virulent and attenuated strains of viruses replicate in host cells in vitro, yet they differ profoundly in their behavior in infected animals, presumably due to differing abilities to penetrate the host mucous membranes, to interfere with host defense mechanisms, and to damage the host. For example, two clones of a recombinant influenza virus had identical surface hemagglutinins and neuraminidases and similar infectivity for the upper respiratory tract, yet one was virulent for ferrets and the other was attenuated (181). These other facets of pathogenicity are as important as replication in overall disease production, but how far virus surfaces are involved is often not as clear as for replication.

INFLUENCE ON MICROBIAL SURFACES OF GROWTH CONDITIONS IN VIVO

Microorganisms grown in vitro may be incomplete as regards all the determinants of virulence, since the genetic basis for virulence may be expressed completely only under the conditions of the test for virulence, namely, during growth in vivo (165, 171 [p. 1]). Loss of bacterial virulence on subculture and increase on animal passage was recognized early, and over the past two decades it has been shown that many bacterial species differ chemically and biologically when grown in animals (165, 171 [p. 1]). Similar conclusions can be drawn from more limited studies on mycoplasmas, fungi, protozoa, and viruses (165, 171 [p. 1]). We should inquire, therefore, into the effects that growth conditions in vivo might have on microbial surfaces and into the evidence for surface differences between microorganisms grown in vivo and in vitro.

In considering bacterial surfaces, we should bear in mind the following fact. The few determinations of growth rate within animal tissues (170) have indicated that multiplication is much slower (one division every 5 to 10 h) than that seen in normal laboratory cultures, suggesting that substrates are limiting. That phenotypic variation of bacterial cell wall structures occurs under limiting growth conditions was shown by continuous culture techniques (52–54, 180). Both gram-positive and gram-negative bacteria and limiting concentrations of Mg^{2+}, PO_4^{3-}, K^+, glucose, and other substrates were used. The doubling times were never greater than 1/h and often 0.1/h under these conditions. Changes were observed in the composition of cell wall teichoic acids, teichuronic acids, mucopeptides, lipopolysaccharides, lipids, and proteins. Though emphasis was on changes in chemical composition of cell walls, there was sufficient information on biological properties to indicate that growth-limiting conditions in vivo might influence the determinants of pathogenicity. For example, the sensitivity of a gram-positive organism to lysozyme – an enzyme contributing to host humoral and cellular defenses (170) – increased with phosphate and ammonia limitation and decreased with Mg^{2+} limitation (54), presumably due to effects on cell wall peptidoglycan structure. The toxicity of cell walls of *E. coli* and *Aerobacter aerogenes* was varied, presumably due to lipopolysaccharide changes, when bacteria were grown under different limiting conditions; with *A. aerogenes*, fast growth produced more toxic cell walls than slow growth under carbon or Mg^{2+} limitation. Glycerol limitation produced the most toxic preparations from *E. coli*, and sulfur limitation yielded the least toxic preparation (52). Similarly, *Pseudomonas aeruginosa* grown in a chemostat with limiting carbon and Mg^{2+} varied in susceptibility to lysis by polymyxin and ethylenediaminetetraacetic acid probably due to changes in outer membrane structure. Even more important in relation to pathogenicity, *P. aeruginosa* grown slowly with limited Mg^{2+} became more resistant to the killing action of intact rabbit polymorphonuclear (PMN) phagocytes and cationic proteins isolated from them. By using serological tests, the increased resistance was correlated with loss of cell wall antigens (59). Similar phenotypic variation of cell wall components having biological properties important in pathogenicity and immunity has been reported for *Bordetella pertussis* (189).

In the examples quoted above, growth-limiting conditions in vitro, which may reflect the conditions in vivo, produced variations in potential determinants of pathogenicity. The conditions of growth in vivo may therefore influence the production of surface components important in pathogenicity, and this has been confirmed by direct observations on bacteria grown in vivo. Hitherto unknown surface determinants of pathogenicity have been revealed by such observations. Examples are antigens associated with prevention of ingestion of *Yersinia pestis* by mouse phagocytes and a cell wall material responsible for resistance of *Brucella abortus* to intracellular bactericidins of bovine phagocytes (170). Similar surface aggressins are also probably responsible for the superior ability of gonococci grown in subcutaneously implanted guinea pig chambers to resist intracellular killing by human phagocytes and extracellular killing by fresh human serum (140, 186). These enhanced resistances to host defense mechanisms were produced partly as a result of selection of gonococcal types with increased resistance to phagocyte killing and partly as a result of phenotypic change, for the resistance to killing by human serum, but not that to phagocyte killing, was lost after a few (one to five) generations in vitro (140). Growth-limiting conditions within the chamber may have contributed to the phenotypic change to serum resistance. After infection with gonococci and the concomitant massive inflammatory response by glucose-using phagocytes, the glucose concentration in the fluid was reduced to very low levels (<0.4 mmol/ml), and gonococcal concentrations remained at 1×10^6 to 5×10^6 organisms per ml for long periods (140).

Other surface changes that take place in bacteria under growth-limiting conditions may enhance uptake of limiting substrates. Permeability may be increased by synthesis of specific transport systems or of cell wall components capable of binding limiting substrates (180) as, for example, the binding of iron by enterochelins (170, 173). Also, cell walls and membranes of bacteria may become more permeable under conditions in vivo and, in the so-called L-phase forms, the cell wall may largely disappear. *B. anthracis* separated by differential centrifugation in saline from the blood of guinea pigs dying of anthrax became swollen and difficult to centrifuge when washed with water. The cells lysed when a trace of ammonium carbonate was added—behavior not shown by organisms grown on four different media in vitro and harvested at four stages of growth (165, 170). A difference in surface properties of *Mycobacterium tuberculosis* isolated from mice and from cultures in vitro was indicated by the ease with which the former was suspended in aqueous media compared with the stringiness of the latter (171, p. 1). Hanks (170) regarded the lack of metabolic activity of *M. tuberculosis* isolated from mice as being caused by loss of essential constitutents through the "leaky" cell wall during isolation. "Leakiness" was considered by Moulder (170) as an important property of intracellular parasites. Large chlamydia that developed from small chlamydia in the vacuoles of mouse fibroblasts lacked cell wall peptidoglycan and were more permeable than the small parent chlamydia (170). The ultimate effect of in vivo conditions on surface properties is seen in the L-phase variants of mycobacteria, streptococci, brucellae, gonococci, and urinary tract bacteria associated with chronic and latent disease (170). How much any increased permeability of cell walls and membranes produced in vivo contributes to pathogenicity is not clear. It seems important for chlamydia but, with bacteria, L-forms do not seem to be significantly different in virulence from ordinary forms. (170).

Viruses are markedly affected by the nature of the host cells in which they are grown (22, 154, 166, 169). The most significant influence of conditions in vivo could occur with viruses that incorporate components of the host cell membranes into their envelopes (40, 154). The envelopes of these viruses can vary according to the cells in which they are grown. Those of viruses obtained directly from infected animals may be different from those of viruses grown on tissue cultures. This will affect their pathogenicity. The considerations apply especially to lipid-coated viruses, which enter cells by fusion between their envelopes and host cell membranes (154). The surfaces of these viruses are lipid bilayers derived from the host cells in which the viruses are grown but with virus-specified proteins and/or glycoproteins inserted (154). In further infection, the enveloped viruses fuse with the membranes of the fresh cells, a specialized example of fusion between biological membranes, requiring a similarity between the interacting membranes for its success (145). Membrane fusion and, so, infection, may be prevented by too great a dissimilarity between the virus envelope formed by budding from one cell type (for example a tissue culture cell) and the plasma membrane of another cell type (for example, a target cell in an animal). In some cases, this may explain the lower virulence of virus produced in tissue culture compared with animal-passaged virus (55). A similar situation may obtain for other obligate intracellular parasites such as chlamydia, which also undergo host-induced modifications of their surface structures (3).

Fungal surfaces are also influenced by environmental conditions in vivo. The morphology of the dimorphic fungi is different in vivo and in vitro. With some exceptions, notably *Candida albicans*, yeast forms occur in vivo and mycelial/arthrospore forms in vitro (171 [p. 251]). The yeast forms seem to be more virulent and immunogenic than mycelial/arthrospore forms and differ from them in cell wall chemistry and antigens (171 [p. 251]). The host nutrients that determine the morphological form in vivo have not been identified, but conversion of saprophytic to parasitic forms has been achieved in vitro (105, 171 [p. 251]). Serum contains a factor that promotes the mycelial form of *C. albicans* that occurs in vivo (13). In addition to selecting the parasitic form of dimorphic fungi, the nutritional conditions in vivo could affect the surface components of any morphological form. Just as bacterial cell wall composition has been changed in vitro (54), the cell wall composition of nonpathogenic yeasts has been changed, by similar procedures, in vitro (120). Also, conditions in vivo seemed to favor the formation of capsule material by *Cryptococcus neoformans* and an increase in resistance to phagocytosis (176).

Most pathogenic protozoa are difficult to grow in vitro, but when this has been achieved, for example, for the trypanosomes, surface structures were affected. This was indicated by changes in morphology, antigenicity, and capacity to infect certain hosts (171, p. 269). Hence, the conditions in vivo preserve the virulence potential of trypanosomes and, as we shall see later, one of the most important aspects of this process is a capacity to change surface antigens during the infectious process.

SURFACE COMPONENTS OF MICROORGANISMS THAT CONTRIBUTE TO ENTRY TO THE HOST: MUCOUS MEMBRANE INTERACTIONS

Although some microbes enter the host directly by vector bite or trauma, most infections begin on the mucous membranes of the respiratory, alimentary, and urogenital tracts, membranes that are protected by moving lumen contents, surface mucus, and often by commensal microorganisms (170, 171 [p. 25, 303]). Early attack on mucous membranes takes at least three forms (161 [p. 174], 171 [p. 25, 203]): attachment and multiplication without significant penetration, as seen in cholera and whooping cough; attachment to, and penetration of, mucosal cells in which they multiply with little or no spread from the initial site, as seen in bacillary dysentry and influenza; and attachment and penetration into the underlying tissues either through or between the mucosal cells as seen in salmonellosis, streptococcal infections, amebic dysentery, and African swine fever.

Methods whereby surface components of microorganisms could contribute to mucosal surface infection and penetration are: (i) by promoting adherence to the host epithelial surface, thus resisting the mechanical flushing action of moving lumen contents; (ii) by aiding competition with the surface commensals for space on the mucosa and for food materials, and by resisting antimicrobial materials (e.g., fatty acids) produced by them; (iii) by resisting humoral and cellular antimicrobial mechanisms in the mucous secretions, not the least being a high or low pH; and (iv) by promoting either the penetration of epithelial cells to cause damage in situ or by breaching epithelial surfaces to facilitate spread of infection to other tissues (161 [p. 171], 171 [p. 25, 203]).

Bacteria

Adherence. Pathogens and potentially pathogenic commensals adhere to mucous membrane surfaces with considerable selectivity. Enteropathogenic *E. coli* adhere to the ileum rather than to the duodenum of pigs and calves and to epithelial cells near the villous tips rather than at the base (6, 171 [p. 25]). In the human oral cavity, *Streptococcus mutans* and *Streptococcus sanguis* adhere to the teeth, *Streptococcus salivarius* to the tongue, and *Streptococcus mitis* to the buccal mucosa (70). Specific interactions between surface components of bacteria and host are responsible for this selective adherence, but only in a few instances are the bacterial components known.

Some, but not all, strains of *E. coli* enteropathogenic for piglets attach themselves to the brush border of the upper small intestine by means of a plasmid-controlled surface protein, the K88 antigen (8, 80, 130, 151, 161 [p. 137], 170, 171 [p. 25]). *E. coli* strains lacking the K88 plasmid were avirulent (170). Colostrum of sows immunized with K88 antigen protected piglets against challenge with a K88-positive strain, which did not then attach to the brush border (158, 170). Immunization with killed, K88-positive, non-enteropathogenic strains also protected piglets from challenge with K88-positive enteropathogenic strains (42). Some piglets inherit the lack of host epithelial receptor for K88 antigen in a simple Mendelian manner: bacteria did not adhere to their intestinal cells, and the piglets were resistant to infection (157, 164).

K88-negative strains can be enteropathogenic in piglets (80, 151, 130), indicating that other surface-adhesive factors may be involved in *E. coli* infections. Strains infecting calves, sheep, and humans also appear to possess "sticking" antigens different from the K88 antigen, the one for the calf strain being designated K99 (23, 57, 76, 121, 136).

S. mutans, which causes dental plaque, adheres to teeth in a two-step process. First, there is a weak reversible association through unknown bacterial components with salivary glycoproteins which first form a pellicle on the teeth (70, 161 [p. 127]). Then, there is a stronger attachment through two sticky glucose polymers (glucans) synthesized from dietary sucrose: a soluble dextran containing predominantly α-1-6 bonds and an insoluble "mutan" containing more than 50% α-1-3 linkages (70, 161 [p. 127]). Mutants with impaired ability to produce the glucans were less able to produce plaque in pathogen-free and gnotobiotic rats

(170), and enzymes that hydrolyzed 1-6 or 1-3 linkages in glucans reduced adherence (70, 161 [p. 127]). Although the physical properties of the glucans are responsible for adherence to the teeth, the glucans themselves are attached to *S. mutans* through a glucosyl transferase, which is bound to a surface polysaccharide containing galactose and glucose. Antiserum against the enzyme prevents adherence (70, 170). The surface products responsible for the adherence of *S. sanguis* to teeth and that of *S. salivarius* and *S. mitis* to various sites in the buccal cavity have not been defined, but a fibrillar coat, possibly of lipoprotein nature, may be involved, at least for *S. salivarius* (67).

Virulent strains of *Streptococcus pyogenes* adhere to the epithelium of the throat by their cell wall M-protein (50, 161 [p. 106, 127], 170). Removal of the M-protein with trypsin or pretreatment with anti-M-protein serum inhibited attachment of streptococci to human epithelial cells (50, 170).

Gonococci adhere strongly to epithelial cells of the urogenital tract in human gonorrhea, in organ culture and in cell suspensions (116, 161 [p. 188], 170, 178, 179), but the surface components responsible are not clear. Pili, present on the putatively virulent Kellogg types 1 and 2 but not on the less virulent types 3 and 4 (175), seem to promote adhesion of in vitro-grown gonococci to tissue culture cells (161 [p. 124], 174, 186). However, both pilated and nonpilated gonococci grown in vitro adhered to the epithelial cells in human fallopian tube (161 [p. 188], 178) and human endocervix (179). Hence, even for gonococci grown in vitro, factors in addition to pili are involved in adherence to epithelial cells. Also, we should remember that in vivo-grown gonococci may have surface components different from in vitro-grown organisms. Additional or different gonococcal surface components produced in vivo were mentioned before in relation to increased resistance to serum and phagocyte killing. In contrast, pili found frequently on in vitro-grown gonococci, are rarely seen on gonococci in urethral pus and scrapings and were only seen spasmodically on those from subcutaneous chambers in guinea pigs (140). However, some pilus material, not necessarily in fibrillar form, must be formed in vivo, since anti-pillar antibodies occur in natural infection (140). Although recently isolated gonococci adhered better to vaginal epithelial cells than after passage in vitro (116), growth of gonococci in subcutaneous chambers in guinea pigs did not enhance their adherence to human endocervix (G. M. Tebbut, D. R. Veale, J. G. P. Hutchison, and H. Smith, unpublished observations). There is still much to be learned about those surface components of gonococci that determine adherence to urogenital epithelium in vivo.

Vibrio cholerae adheres to the epithelium of the small intestine of man and experimental animals (63, 132, 161 [p. 137, 154], 162, 171 [p. 25]). The surface component responsible for adhesion is not known. There is a correlation between motility and adhesion of strains, but a surface adhesion component that is separate from the flagella seems to be involved (63, 75, 162). *Salmonella typhimurium* adheres to the epithelial cells of mice (161 [p. 120], 171 [p. 25], 177), *Clostridium perfringens* attaches to the intestinal villi of pigs (7), and streptococci and staphylococci adhere to bovine udder (64), but as yet the surface components concerned are not known. Mycoplasmas attach to and attack epithelial cells, particularly those in the respiratory tract (74, 171 [p. 217]). Some surface components concerned may be proteins since trypsin treatment reduced the adhesion (82), but others may be carbohydrates, glycoproteins, or glycolipids (149, 161 [p. 143]).

Competition with commensals. Competition and antagonism between the indigenous bacterial flora of mucous surfaces and potential pathogens were indicated by the survival of pathogens introduced after antibiotic treatment had removed some indigenous bacteria (161 [p. 116, 120], 170). In many instances, this competition and antagonism has been confirmed by mixed-culture experiments in vitro and in gnotobiotic animals (161 [p. 116, 120], 170). However, with few exceptions, neither the antibacterial mechanisms of the commensals nor those that allow pathogens to survive are clear (161 [p. 116, 120], 170). Hence, it is too early to identify the role of surface components of pathogens in survival on mucous surfaces. Stronger adherence mechanisms would allow pathogens to compete successfully for space; e.g., gonococci adhered more strongly to human vaginal cells than *Lactobacillus acidophilus* and other commensals (116). However, knowledge of the surface components responsible for adherence of pathogens and commensals is too scanty to allow discussion of relative strengths of adhesion. Similarly, successful competition for food materials might be brought about by the pathogen possessing superior permeases or surface chelating compounds (see next section), but we do not have sufficient knowledge to discuss the matter. Also, surface components of pathogens could confer resistance to the action of bactericidins or toxic metabolites (e.g., fatty acids) produced by commensals such as the resistance mounted by *Shigella flexneri* in gnotobiotic mice against the activities of *E. coli*. (161 [p. 47, 120], 170). Again no specific surface component has been implicated, but it is possible that lipopolysaccharides provide a barrier to toxic fatty acids (188).

Resistance to host defense mechanisms in mucus. Host secretions from mucous surfaces contain nonspecific and immunospecific antibacterial substances (161 [p. 127, 154], 170, 171 [p. 1, 25]). Also, large numbers of phagocytes are extruded, a process that can be mediated by immune responses (170). Bacteria probably survive these host defense mechanisms by processes similar to those used to inhibit humoral and cellular defenses in the blood and tissues. The surface components concerned are described in a later section.

Penetration. Light and electron microscopy of mucosal invasion in animals, coupled with investigations both in vivo and in vitro, has increased our knowledge of some penetration processes (62, 161 [p. 165], 171 [p. 25]). However, the bacterial products involved – be they surface bound or extracellular – are obscure, except perhaps for those of *S. flexneri*. These bacilli enter and multiply in intestinal epithelial cells of humans and primates. They rarely penetrate beyond the epithelium to the lamina propria but spread laterally, producing necrosis and death of patches of cells, which leads to the ulcers and diarrhea characteristic of dysentery (171 [p. 25]). The ability to penetrate intestinal epithelial cells is the paramount virulence attribute, and mutants lacking it are avirulent (62, 161 [p. 165], 171 [p. 25]). Mucosal invasion by *Shigella* has been investigated in primates and guinea pigs, as well as their ability for cell penetration in HeLa cell monolayers and in the conjunctivae of guinea pigs and rabbits (62, 161 [p. 165]). Genetic experiments indicate that the surface O antigen of *S. flexneri* may be responsible for penetration. Intergeneric hybridization of *S. flexneri* and *E. coli* produced *S. flexneri* derivatives expressing either *E. coli* O25 or O8 somatic antigens. The behavior of these hybrids with the parent *S. flexneri* and *E. coli* strains was compared first in the conjunctivitis test and then in mucosal invasion of guinea pig intestine. The results indicate that an *N*-acetylglucosamine-rhamnose-rhamnose repeat unit in the O antigen of *S. flexneri* may be the determinant of penetration. Replacement of this unit by one containing D-mannose (that of *E. coli* O8) resulted in a hybrid unable to enter cells; but when a unit containing rhamnose was substituted (that of *E. coli* O25), the hybrid retained the ability to penetrate (62, 161 [p. 165]).

Salmonella species pass through the small intestine into the lamina propria where they spread to other tissues, possibly within macrophages (171 [p. 25]). Penetration of *S. typhimurium* has been much studied in rats, mice, and guinea pigs, in the rabbit ileal loop, and in HeLa cells (62, 115, 161 [p. 174], 171 [p. 25], 177). The main path of the penetrating bacteria is through epithelial cells, although passage between them also occurs. The initial penetration process is clear: degeneration of the microvilli when a bacterium approaches within 350 μm, degeneration of the apical membrane and cytoplasm, vacuolation and ingestion of the bacterium, and shrinkage of the vacuole until the bacterium is surrounded by a single membrane of electron-dense material (161 [p. 171]). However, neither the bacterial products responsible for the initial penetration nor the processes whereby the bacterium escapes from the epithelial cell through the basal plasmalemma are known. Work with strains having different O antigens suggests that these surface antigens may influence penetration, but not profoundly (62, 161 [p. 171], 177). Perhaps an extracellular product is involved, because the cell microvilli degenerate before being touched by the bacterium. *Salmonella enteritides* seems to penetrate through the Peyers patches (161 [p. 170]), but whether surface components promote this process is not known.

Gonococci also seem to penetrate mucosal surfaces of the genital tract through columnar cells by an engulfment process akin to phagocytosis (161 [p. 188]). The intracellular gonococci pass deeper into the cell and laterally into the intercellular space and adjacent cells. The bacterial products that assist the penetration are not known.

Viruses

Although surface components of viruses are probably important in promoting the early stages of virus disease at mucous surfaces, there are as yet few well-proven examples.

Adherence. The mechanisms whereby viruses penetrate the mucus blanket are not known (166, 171 [p. 303]), but degradation of some mucus constituents by the surface neuraminidases of the myxoviruses and paramyxoviruses (126) is possible. Adherence to mucous surfaces is easily explained for those viruses that infect epithelial cells such as influenza virus, rhinoviruses, poliovirus, and foot and mouth disease virus (171 [p. 303]), since attachment is the first stage of the replication process and, of course, envelope components are involved. The close adherence that precedes infection can often be seen in electron micrographs (166). However, for viruses that seem to penetrate the mucosa without establishing infection in the membrane itself, as, for example, rinderpest and African swine fever (171 [p. 303]), we do not know whether adherence occurs or whether surface components are involved in mucosal invasion.

Competition with commensals. In cell culture and in animals bacteria and fungi, as well as other viruses, affect virus infections by producing interferon (60). Also, mycoplasmas, notable inhabitants of mucous surfaces, inhibit the replication in cell culture of viruses such as herpesvirus (78) and measles virus (153). If such microbial interactions occur on mucous surfaces, as is likely, virulent viruses must be able to deal with them, but the role of virus surface components is not known.

Resistance to host defense mechanisms in mucus. Mucus can have antiviral activity, sometimes merely due to a low pH. The destructive effect of the acid pH of the stomach on rhinoviruses and influenza virus is well documented (58). Viral inhibitors have been found in homogenates of lung and intestinal mucosa and may be excreted in the mucus (166). Bile contains viral inhibitors (106), which dissociate enveloped viruses (58). Viruses are also destroyed by phagocytes extruded onto the mucous surface (171 [p. 303, 333]). Surface components are probably involved in survival of virulent viruses against these defense mechanisms, but this has yet to be proved.

Penetration. When penetration through the mucosa occurs without infection of the epithelial cells, the mechanisms involved and the role of surface components are not known. When epithelial cells are infected, virus surface components take part in attachment and penetration and also in release from the infected cell; but whether they are involved in deeper penetration into the tissues after the preliminary infection is not clear.

Fungi

What products of fungi are responsible for adherence to mucous surfaces is not known, although *C. albicans* adheres less strongly than streptococci to oral cells (114). Antifungal processes operative on these surfaces include inhibitory activity by bacterial commensals and fungistatic materials such as the fatty acids in the teat secretions of domestic animals (171 [p. 251]). Surface components of virulent fungi probably take part in overcoming these antifungal mechanisms at body surfaces, but there is no proven example.

Protozoa

Many protozoa are injected into the host by vector bite, but for those that invade across mucous surfaces, little is known of the mechanisms concerned. The interaction of *Entamoeba histolytica* with epithelium and its invasion of gut mucosa has received some attention (171 [p. 269], 176). When entamebae invade the lamina propria of guinea pigs, PMN phagocytes are destroyed, epithelial cells are shed, and vascular damage occurs, probably as a result of PMN destruction (176). To what extent surface components or extracellular enzymes of the pathogen are responsible for the changes is not known. In lysing tissue culture cells, entamebae transfer lysosomes, which protrude from their surface, into the cells by a trigger mechanism that involves rupture of the cell membrane (171 [p. 269]). Surface-to-surface interactions occur, but their precise nature is not clear. Lysosomal transference may not operate in vivo since, during mucosal invasion, mesenchymal cells in direct contact with entamebae are unharmed, whereas free PMN phagocytes are destroyed by an unknown mechanism (176).

SURFACE COMPONENTS OF MICROORGANISMS THAT CONTRIBUTE TO MULTIPLICATION IN VIVO

An essential attribute for pathogenicity is an ability to multiply in the environment of the host tissues. The more rapid the rate of multiplication, the more likely is infection to be established despite the activity of the host defense mechanisms. For nonviral pathogens, the requirements for multiplication are low-molecular-weight nutrients and minerals in the host tissues and the appropriate Eh and pH. Thus, surface components of pathogens might affect the issue by promoting or inhibiting the inflow of essential nutrients such as iron (170, 173) from the host tissues. Viruses, on the other hand, are obligate parasites and require the cooperation of the intact host cell at all stages of replication: attachment, penetration, uncoating, provision of appropriate metabolic conditions for synthesis of virus components, assembly, and release. The features of the host cell and its environment that control these processes have been called "replication factors" (166), and they are the virological counterparts of the growth conditions required by other microorganisms. Surface components of viruses can complement or antagonize the host cell processes.

Bacteria

There is little information about the influence of surface components on the uptake of nutrients in vivo; the best-documented case is that of supply of iron.

Iron is essential for bacterial growth and, to ensure the supply of iron, pathogenic and nonpathogenic species synthesize chelators of iron called siderochromes, especially under conditions of iron deficiency (101, 131, 171 [p. 75], 173). The siderochromes differ in chemical structure. Mycobactins, formed by mycobacteria including *M. tuberculosis*, are aromatic dihydroxamates (101); *E. coli* and *A. aerogenes* produce 2,3-dihydroxybenzoic acid and 2,3-dihydroxybenzoyl serine (122, 173); *S. typhimurium* and *E. coli* make enterochelin, a cyclic trimer of 2,3-dihydroxybenzoic acid (122, 173). Many siderochromes are excreted into the medium to chelate iron, which then enters the cell as a complex with the siderochrome (122, 173). However, others are found in the surface layers: mycobactin is a lipophilic molecule fixed in the cell wall of mycobacteria and it transports iron across the lipoid layers into the cell (114). The siderochromes that are excreted may also be present in the surface layers of the bacteria and play some role in entry of iron.

What part do siderochromes play in pathogenicity? Although they are produced by nonpathogens as well as pathogens and by avirulent as well as virulent strains (122, 123), they may be one of a complement of virulence factors needed to produce disease, for the following reason. The iron-binding compounds transferrin and lactoferrin are present in serum, mucus, and milk. Alone, and sometimes in conjunction with antibody and complement, they seem to prevent the growth of many pathogens (*C. perfringens, Y. pestis, Yersinia septica, E. coli, P. aeruginosa*) by denying them essential iron. In some cases, this may result in inhibition of ribonucleic acid (RNA) synthesis (20, 122, 123, 170). When these organisms are inoculated into animals along with iron compounds, their pathogenicity is increased enormously. Although iron can inhibit other host antibacterial mechanisms, such as basic proteins of phagocytes (170), myeloperoxidase, (173), and hydrogen peroxide (94), at least part of its action seems to consist of saturating the transferrin and lactoferrin so that it is then available for bacterial growth. Thus, in the normal situation where external iron is not supplied, the ability to compete successfully with transferrin or lactoferrin for iron may be as essential to a pathogenic organism as possession of any of its other determinants of pathogenicity. Indeed, the virulence of an attenuated strain of *E. coli* for mice was enhanced by injecting it with a preparation of an iron chelator from a virulent strain (152). Also, presumably due to the superior effectiveness of their siderochromes, the virulence of virulent gonococcal strains for the chicken embryo, unlike that of attenuated strains, was not substantially increased by iron compounds (139).

Viruses

Virus replication (58) cannot be described in detail. Surface components are essential in the initial processes of attachment and penetration by viropexis (endocytosis) or fusion, in uncoating and assembly of virions, and, finally, in their release (40, 154). At all stages, there must be complementation with the appropriate "replication factors" of the host cell. Already, we have discussed how, on release of enveloped viruses, host cell membrane components are incorporated into their surfaces and influence the early stages of subsequent infection. Without complementation by the host cell, replication does not proceed, and variation of this complementation is one explanation for host and tissue specificity in virus infections (169). The importance of virus surface components in promoting replication and therefore pathogenicity is illustrated by the following examples. When vesicular stomatitis virus lost a surface glycoprotein on treatment with bromelain or Pronase, it became spikeless and noninfectious, but, when treated with the purified glycoprotein, its infectivity was restored (15). Virulent strains of poliovirus adhere to the receptors of primate nerve tissue more strongly than avirulent strains (166), suggesting subtle differences in the viral surface components responsible for virulence. Structural differences in the capsid proteins of a neurovirulent (Mahoney) and an attenuated (1 SC2ab) strain of poliovirus have been noted (166). Chromatographic differences were detected among all three peptides obtained by trypsin treatment of the two capsid proteins; one peptide from the attenuated strain also differed in that it was unstable when treated with sodium dodecyl sulfate. Presence of surface components of Newcastle disease virus that promote fusion with host cell surfaces seems to correlate with the virulence of the strains (146). Differences in surface structure of virulent and attenuated strains of Newcastle disease virus were also indicated by a hemolytic system (5). Finally, it should be remembered that antibodies directed against surface components that promote virus entry to cells, such as the hemagglutinins of influenza virus and the capsid proteins of poliovirus, seem to be important in protecting against disease (112, 183).

Fungi

The influence of nutritional conditions in vivo on morphological form and, therefore, on surface components of fungi has been described in a previous section. The role of surface components in promoting multiplication in vivo is not clear, but *C. albicans* at least may possess siderochromes (49).

Methods for measuring mycelial growth in animals have been inaccurate (10), but recently a more reliable method has been introduced which depends on assay of cell wall chitin (107). The method was adapted from one used for fungal infections in plants (150) to measure the growth of *Aspergillus fumigatus* in normal and immunosuppressed mice. Mycelial growth localized in the kidney and was more rapid in immunosuppressed animals. The chitin assay has also been used for following the growth in mice of virulent and attenuated strains of *C. albicans* (E. Gibb and L. O. White, unpublished data).

Protozoa

Information on the nutrition of protozoa in vivo is available (171 [p. 269]), but there is none on the role of surface components.

SURFACE COMPONENTS OF MICROORGANISMS THAT CONTRIBUTE TO INTERFERENCE WITH HOST DEFENSES

Details of host defense against infection and microbial interference with this defense can be found elsewhere (4, 166, 167, 171 [p. 1, 75, 333]). In summary, host defense can be humoral, cellular, or a combination of both, acting nonspecifically against any pathogen or specifically against a single invading species when an immune response is illicited. During the first few hours within the host, pathogens must cope with antimicrobial mechanisms already present in the tissues and then with phagocytes (short-lived PMN phagocytes and long-lived mononuclear [MN] phagocytes) mobilized by inflammation soon after the tissues are irritated. In this primary lodgement period of infection (170), the antimicrobial mechanisms of the host are weighted against the few invading microbes, and many infections are eliminated here. If microbes survive at the primary site, spread of infection is opposed by MN phagocytes fixed in the lymph nodes, spleen, and liver. Survival in spite of the activities of these phagocytes results in acute disease. Sometimes the host is killed, but usually the disease subsides because, a few days after infection, there is a developing immune response. This response not only increases the efficiency of the phagocytic defense considerably but also provides antibodies capable of directly neutralizing viruses or microbial products that are important in the disease process. Eventually, most infections are eliminated by these immune reactions, and only in chronic disease and carrier states is long-term survival achieved.

Pathogens are unique among microorganisms in being able to overcome the host defense mechanisms. They do so by producing compounds or inducing processes that inactivate host defense mechanisms, resist their action, or even prevent their appearance. The bacterial products having this biological effect have been termed aggressins, auxiliary pathogenic factors, and impedins (171 [p. 75]), and these names could be applied to similar products formed or induced by other microorganisms. Some of the aggressins are extracellular, but many are surface components. They can act by: (i) conferring resistance to humoral antimicrobial agencies in blood, milk, and other body fluids; (ii) stopping mobilization of phagocytes by inflammation: (iii) hindering contact with phagocytes; (iv) preventing ingestion by phagocytes; (v) interfering with intracellular killing by phagocytes, leading to short-term survival in PMN or long-term survival in MN cells; and (vi) inhibiting the immune response or reducing its effectiveness.

Bacteria

Interference with humoral defenses. Resistance to humoral bactericidins is characteristic of virulent strains of many species including *B. anthracis, Staphylococcus aureus, Leptospira* spp., *Enterobacteriaceae*, and *B. abortus* (170). The aggressins responsible for the resistance are known only in a few instances, but some are surface components. The lipopolysaccharides of gram-negative organisms interfere with humoral bactericidins (161 [p. 336], 188); capsular poly-D-glutamic acid of *B. anthracis* interferes with the bactericidins of horse serum (170). The surface K antigens of *E. coli* infecting the urinary tract interfere with the lytic action of antibody and complement: strains producing these acidic polysaccharides establish infections in the kidney rather than being confined to the bladder. Different K antigens vary in their inhibiting activity, but the amount of K antigen produced also seems important (117, 133, 170). Spread of *E. coli* in the blood stream seems to be controlled by other factors, since bacteremic strains do not have excessive amounts of K antigens (117). A cell wall component of *B. abortus* containing protein, carbohydrate, formyl residues, and about 40% of lipid interferes with the bactericidins in bovine serum (170).

Gonococci grown in vivo resist the killing action of human serum, and this resistance is probably important in the pathogenesis of gonorrhea (140). The resistance is almost certainly due to a surface component, as yet unknown. Gonococci collected directly from urethral pus were more resistant to human bactericidins than gonococci that had been subcultured on laboratory media, unless the latter contained extract of human prostate (171 [p. 75]). As described before, gonococci examined directly after growth in plastic chambers implanted subcutaneously in guinea pigs were similarly resistant to killing by human serum. This resistance was lost after a few generations in laboratory media, suggesting that the in vivo conditions influence the bacterial phenotype (140). Recently, resistance of Kellogg types 1 and 2 gonococci to killing by human serum was increased by serial passage in serum (140), but whether such organisms were as resistant as those from in vivo sources is not yet clear.

Serum, tissue extracts, and body fluids contain mycoplasmacidal materials (171 [p. 217]), and virulent mycoplasmas must withstand these materials to produce disease. Whether surface components are concerned in this resistance is not known.

Interference with mobilization of phagocytes. Virulent staphylococci multiply in mice more rapidly than avirulent strains and produce more severe lesions by suppressing the inflammatory response (170). A cell wall mucopeptide has an anti-inflammatory activity and acts by preventing release of kinins (170). The significance of the mucopeptide in human infections is not known, but in mice it seems more important in local (skin) infections than in systemic ones (48).

Prevention of contact with phagocytes. Chemotaxis has been inhibited in vitro by cell wall fractions from tubercle bacilli and staphylococci (170), but whether these materials are active in infection is not known.

Interference with ingestion by phagocytes. Once ingested, many bacteria (salmonellae, pneumococci, anthrax, and plague bacilli) are usually destroyed and digested. Resistance to ingestion is the main virulence mechanism of these bacteria and in many cases surface and capsular products are responsible (Table 1). Gonococci grown in vitro and in vivo resist ingestion by human phagocytes to some extent but not completely. Intracellular gonococci are seen in urethral pus and, in phagocytosis tests, in vivo- and in vitro-grown, putatively virulent, Kellogg types 1 and 2 gonococci were ingested, although not as readily as attenuated Kellogg types 3 and 4 gonococci (186). Since the virulent types possess pili, several authors believe that pili are responsible for resistance. However, Swanson and his colleagues have described a nonpilated strain resistant to phagocytosis and think that association of gonococci with human PMN phagocytes is governed mainly by a surface protein called "leukocyte association factor." This factor is not exclusive to either virulent or attenuated strains (186). Pili were considered to have a minor role in preventing association of gonococci with human phagocytes but a major one in preventing ingestion by mouse MN phagocytes (186).

TABLE 1. *Bacterial surface components that prevent ingestion by phagocytes*

Pathogen	Capsular or surface component	Reference
Pneumococci	Polysaccharide	170, 171[a]
Meningococci	Polysaccharide	170, 171
Streptococci	M-protein, hyaluronic acid	170, 171
Staphylococci	Mucopeptide	170, 171
Bacillus anthracis	Poly-D-glutamic acid	170, 171
Yersinia pestis	Fraction 1: polysaccharide-protein complex	170, 171
Enterobacteriaceae	Complete O antigens	170, 171
Salmonella typhi	Vi antigen (poly-*N*-acetyl-D-galactosaminouronic acid)	170, 171
Escherichia coli	Acid polysaccharide K antigens	170, 171
Treponema pallidum	Possibly hyaluronic acid	103
Pseudomonas aeruginosa	Slime	46, 170
	Possibly leucocidin	159

[a] When reference 171 is cited, see p. 1 and 75.

Mycoplasmas can be ingested and killed by phagocytes, and sometimes opsonization plays a part (19, 138, 147, 161, 171 [p. 217]). Virulent mycoplasmas must resist ingestion, and surface materials may be responsible in some cases (19). Whether capsular molecules such as the galactan of *Mycoplasma mycoides* (161) can act in the same way as pneumococcal polysaccharides is still not clear. In the case of rickettsiae, surface antigens appear to prevent ingestion by PMN phagocytes (14).

There has been little work on the relationship between interference with ingestion and chemical structure of surface components. Resistance of *E. coli* to phagocytosis by mouse PMN phagocytes seems to depend on a complete polysaccharide side chain in the cell wall antigen: a mutant lacking colitose was more susceptible to phagocytosis than the wild type, and a mutant lacking galactose, glucose, *N*-acetylglucosamine, and colitose was even more susceptible (170). Similarly, complete sugar sequences in the core and polysaccharide side chain of the O antigens are necessary for full resistance in phagocytosis of *S. typhimurium*; the tetrasaccharide sequences abesquosyl-mannosyl-rhamnosyl-galactose have been suggested as the determinant group, acetyl and glucosyl groups being less important (170). Although resistance to phagocytosis usually correlated with virulence for mice, small changes in O antigen structure can influence virulence without affecting phagocytosis (170, 185). The antiphagocytic moiety of the M-protein of streptococci seems to be separate from that which determines serological specificity, but its chemical structure is not known (61).

The mode of action of surface aggressins in inhibiting ingestion is still not clear. They may act by purely mechanical means or by inhibiting adsorption of serum opsonins, as seems to be the case for *B. anthracis* and staphylococci (170). Also, they may render the bacterial surface less foreign to the host; for example, the M-protein of streptococci has antigenic groups similar to those of host tissue components (170).

Prevention of intracellular digestion by phagocytes. Some bacteria can survive intracellularly in PMN phagocytes or MN phagocytes. PMN phagocytes live only for a few days in vivo. Ability to resist their bactericidins can, therefore, contribute to bacterial survival and dissemination in the early phases of disease, since surviving bacteria will be liberated when the PMN cells die. This process seems to occur in brucellosis, plague, staphylococcal infections (170), and gonorrhea (186). In cases where intracellular survival has been probed, microbial surface components seem to be involved.

Virulent strains of *B. abortus* survived and grew in the phagocytes (predominantly PMN with some MN) of bovine "buffy coat," whereas attenuated strains were gradually destroyed (170). The intracellular survival of the virulent strains was due to production of a cell wall substance that interferes with the bactericidal mechanisms of the phagocytes. This occurred when the organisms were grown in vivo and under simular conditions in vitro. Thus, virulent brucellae from infected bovine placental tissue or from cultures in laboratory media supplemented by bovine placental extracts (170) survived better intracellularly than the same strain grown in unsupplemented laboratory media. Cell wall preparations of the organisms from infected bovine placenta and from the supplemented media inhibited the intracellular destruction of an avirulent strain of *B. abortus* (170). The surface antigen that appeared to be responsible for inhibition of the phagocytic bactericidins was removed from *B. abortus* grown in supplemented medium by washing with an ether-water mixture (170). This antigen was serologically different from the cell wall material described previously, which interfered with the humoral bactericidins of bovine serum (170). How it acts is not known.

Staphylococci grown in rabbits were more resistant to killing by rabbit PMN phagocytes and their extracts than staphylococci grown in vitro (170). This resistance appeared to be due to a surface layer of host protein, which may be deposited as a result of the action of free and bound coagulase (170).

Although some gonococci are killed within human PMN phagocytes, others survive and grow intracellularly: this occurs in urethral pus and in phagocytosis tests in vitro (186). When grown in vitro, a putatively virulent strain survived and grew better than an avirulent strain within human phagocytes (186). It was even more resistant to intracellular bactericidins after adaptation to growth in guinea pig chambers, which selected phagocyte-resistant organisms from the original population (140, 186). A surface component is probably responsible for the resistance to intracellular killing by PMN phagocytes, but it has yet to be identified.

Turning now to intracellular survival and growth in long-lived MN phagocytes, I shall deal with the manner in which surface components of typical intracellular pathogens, brucellae, tubercle bacilli, and leprosy bacilli, contribute to bacterial survival for long periods within the host and produce chronic disease.

Brucellae grow in bovine macrophages (170). The bactericidal mechanisms of these macrophages may have been inhibited by the same cell wall material that has been shown to inhibit the bactericidins of the mixed PMN and MN phagocytes of "buffy coat" (see above).

M. tuberculosis (and *Mycobacterium microti*, which causes vole tuberculosis) and *Mycobacterium lepraemurium* appear to resist intracellular bactericidins by different mechanisms. In mouse peritoneal macrophages infected with virulent *M. tuberculosis* and *M. microti*, the lysosomes did not discharge into phagosomes, which contained intact bacteria (170). This inhibition of discharge appears to be the foremost pathogenic mechanism, although tubercle bacilli treated with antibody to allow granule discharge were then resistant to the released enzymes (9). The way in which the discharge is prevented is not clear; material in electron-transparent layers surrounding the bacilli may be involved (170), but secretion of extracellular cyclic adenosine 5'-monophosphate into the vacuoles has also been implicated (170). With *M. lepraemurium*, lysosomal discharge into the phagosomes occurred normally in mouse peritoneal macrophages and in rat fibroblasts, but the pathogen was resistant to this discharge (170). The resistance appears to be due to a surface material, a type C mycoside (peptidoglycolipid), which was isolated from the livers and spleens of mice infected with *M. lepraemurium* (170).

When mycoplasmas are phagocytosed after opsonization, they seem to be killed (19, 138, 147, 171 [p. 217]), although some resist the killing action better than others (27), and surface components may be involved. Intracellular survival of rickettsiae in macrophages has been clearly demonstrated in cell culture and may be important in the pathogenesis of rickettsial diseases although, in animals, it still has to be demonstrated convincingly (96, 194). Again, surface components may help the organisms to resist intracellular killing.

Interference with the immune response. Bacteria can interfere with the effectiveness of the immune response by two methods – antigenic shift and direct suppression of the response. Surface components are involved in the former and probably in the latter.

In antigenic shift, surface antigens are changed so that existing potentially protective antibodies formed in response to the original surface antigens become ineffective. This shift seems to occur in some bacterial diseases and during persistence of potentially pathogenic commensals on mucous surfaces. The surface antigens of *Borrelia recurrentis* seem to change during relapsing fever (170). Similarly, antigenic variation and selection occur in infections of *Campylobacter fetus* in cows (31) and *V. cholerae* in gnotobiotic mice (124). On mucous surfaces, *E. coli* serotypes inhabiting the human intestine change periodically (30), and antigenic variation of oral streptococci has also been detected (17).

Suppression of both antibody and cell-mediated immune response occurs in bacterial infection (95, 163); examples are seen in *P. aeruginosa* infections, mycoplasmal infections, tuberculosis, leprosy, and syphilis (19, 163, 170). To what extent surface components of the pathogens are involved is not clear (163). Antibody response was decreased by membrane fragments from group A streptococci (163). The membranes of *M. arthriditis* were immunosuppressive in rats, as was the capsular polysaccharide of *Klebsiella pneumoniae* in mice (163). Endotoxins can also be immunosuppressive (163, 170), although certain cell wall preparations containing them appear to have an adjuvant effect on vaccines (163, 170).

Viruses

Considerable information is available on host defense against viruses, but there is little information on how virulent strains overcome this defense (16, 22, 58, 166, 171 [p. 333]). Consequently, the role of virus surface components is obscure, and one can only outline main areas where they might be implicated.

Interference with humoral defenses. Many tissue extracts and sera contain nonspecific viral inhibitors and others can be induced by virus attack (166), but the role of surface components in any differential resistance of virulent and attenuated virus strains has not been investigated.

Interference with cellular defenses. Antiviral factors such as interferon or host nucleases may be present or induced in any cell the virus attacks. However, the major cellular inhibitors of virus infection are phagocytes of the reticuloendothelial system, which act nonspecifically but are strengthened in their action by immunization (16, 22, 58, 166, 171 [p. 333]).

Interferon is produced by phagocytes as well as by other cells, and its induction and inhibitory activity vary with the inducing virus and host cell (60). A connection between the virulence of a virus strain and its capacity to induce and resist interferon has not been established clearly (88, 128, 166); whether or not viral surface components affect virulence through this agency is a matter of conjecture. Although viruses appear to produce antagonists to interferon in vitro (166), it is not known whether these antagonists are produced in vivo, have a role in pathogenicity, or are connected with virus surface components.

The inflammatory response is not seen in slow-virus infections, so presumably such viruses do not stimulate the response. Depression of chemotaxis of monocytes after infection with influenza and herpesviruses has been noted (98), although the part that surface components may play is not known. Some viruses are resistant to phagocytosis by macrophages; others are ingested and are either destroyed or allowed to replicate (171 [p. 333]). Ability to replicate within macrophages is sometimes related to the virulence of strains (4, 39, 56, 91, 155, 166, 171 [p. 333]). Surface components to viruses will be involved in contact and ingestion by phagocytes and could affect virulence by resisting or promoting ingestion, but differences in surface components related to differences in ingestion have not been detected between virulent and avirulent strains. Also, within phagocytes certain viral surface components could confer resistance to the killing action of host enzymes released into phagosomes, but again authenticated examples are absent. Finally, the killing mechanisms of phagocytes might be inhibited by toxic effects of virus infection (166), and indeed inhibition of the ability to ingest bacteria has been noted after virus infection of phagocytes (89, 166). It will be seen in the next section that, in some cases, surface components of viruses are cytotoxic. These conjectures about the role of surface components in resisting the action of macrophages apply equally well to phagocytosis of viruses by PMN phagocytes (166) and to ingestion and replication in lymphocytes (43).

Interference with the immune response. The immune response strengthens the host defence against viruses. Antibodies complex with surface components essential for host cell penetration, or opsonize viruses or virus-infected cells for destruction by phagocytes. They also increase the destructive effect of macrophages and lymphocytes toward viruses and virus-infected cells (118, 135, 171 [p. 333], 190). Viruses could interfere with the effectiveness of the immune response by being "bad" antigens in the first place, or by antigenic shift. They could also infect the cells responsible for antibody formation and cell-mediated immunity and impair their functions. Surface components would be involved in the first processes but not necessarily in the others. Virus strains vary in their ability to evoke antibody (166) and "slow" viruses stimulate hardly any antibody production (166). We do not know why some antigens are "good" and others "bad" (167), but viruses with host cell membrane constituents in their envelope proteins may be more "hostlike" and therefore "bad" antigens. However, virulent strains of virus do not appear to be less immunogenic than attenuated ones (166). Drifts and shifts of surface antigens occur for respiratory and other viruses between epidemics, facilitating infection of new hosts, but as far as I am aware, they have not been detected during chronic infection of one host. Suppression of antibody production and cell-mediated immunity by virus infection is well documented (119, 166), but the mechanisms are obscure. As for inhibition of the function of macrophages, the cause might be the cytotoxicity of viral surface components on cells responsible for the immune response.

Fungi

Serum and tissue extracts contain nonspecific fungicidal materials, some of them complement dependent (44, 113, 171), but whether virulent strains of fungi resist these materials better than avirulent strains is not known, nor is it known if surface components are involved. Phagocytes kill fungi (108–110, 171 [p. 1, 251]), although their effectiveness varies: peritoneal PMN phagocytes, but not lung macrophages, killed noncapsulated *C. neoformans*, (21) and PMN phagocytes, but not macrophages, killed *Histoplasma capsulatum* (85, 171 [p. 1, 251]). Some fungi resist ingestion by phagocytes and, for *C. neoformans*, the surface aggressin responsible is known. This is a capsular polymer containing uronic acid, and the larger the capsule the greater the resistance to ingestion (102, 125, 171 [p. 1, 251]). Many fungi resist intracellular killing. *C. albicans*, *Coccoides immitis*, and *H. capsulatum* survive and grow within MN phagocytes (137, 171 [p. 1, 251]) and so do any *C. neoformans* that are ingested (44). Conidia of *A. fumigatus* survived for long periods in human PMN and MN phagocytes (109) and, in myeloperoxidase-deficient PMN phagocytes, *C. albicans* survived, whereas less pathogenic fungal species were destroyed (108). Fungal surface components are probably responsible for this resistance to intracellular killing, but none has been recognized and identified. Differences in cell wall composition were detected between virulent and avirulent strains of *Blastomyces dermatitides* (33), but whether such differences are connected with different susceptibility to phagocytes is not known.

Protozoa

Lysis by antibody and complement is the main humoral defense mechanism (72, 171 [p. 269]), and antigenic shift is a process adopted to sidestep it (171 [p. 269]). Antigenic shift has been best studied in the trypanosomes (37). They have a surface coat composed of a closely packed monolayer of a series of glycoproteins that are expressed sequentially during a persistent infection. Glycoproteins purified from trypanosome populations taken at successive times from the start of infections in animals had the same molecular weight (about 70,000) and carbohydrate composition (about 6% by weight) but differed profoundly in amino acid composition and sequence. The number of changes that can take place from one clone of inoculated trypanosomes is not known, nor is the mechanism of surface replacement known.

Protozoa are also ingested and killed by PMN and MN phagocytes, especially those from immunized hosts (79, 93, 104, 171 [p. 1, 269], 176). Some protozoa such as *Plasmodia* spp. may need to be opsonized by antibody before ingestion (171 [p. 1, 269]), and others such as *Toxoplasma gondii* require similar treatment before being killed intracellularly (93); in either case, antigenic shift would interfere with the defense mechanisms. Sometimes, protozoa seem to survive and grow intracellularly in phagocytes. This occurs with *Leishmania* spp. (25, 171 [p. 1, 269]), *Trypanosoma cruzi* (104) and *T. gondii* (92); the latter prevented granular discharge in a manner similar to tubercle bacilli. However, no protozoal product – surface or otherwise – has yet been identified as responsible for resistance to ingestion or digestion by phagocytes.

Immunosuppression occurs in protozoal disease (163), but whether surface components are involved is not known.

SURFACE COMPONENTS OF MICROORGANISMS THAT CONTRIBUTE TO HOST DAMAGE

Microbes damage tissues by two main processes: directly by the production of toxins (1, 161, 171 [p. 113, 129, 359]) and indirectly by evoking immunopathological reactions (58, 171 [p. 157, 383]). As regards toxins, those formed extracellularly by bacteria, fungi, and protozoa (1, 171 [p. 113, 129, 251, 269]) and those induced in cells by viruses (11, 171 [p. 359]) probably play the major role in producing host damage, and these substances fall outside the scope of this review. Whether or not toxic surface components, such as the endotoxins of the gram-negative bacteria or the penton of adenovirus (166, 171 [p. 359], 188), cause host damage depends on the extent to which they are liberated during infection. The point is well illustrated by reference to endotoxins. Endotoxins obtained from the cell walls of different gram-negative bacteria by treatment with trichloroacetic acid or warm aqueous phenol produce similar toxic manifestations – pyrexia, prostration, diarrhea, and death. In some infections, they are also liberated from the cell wall of the infecting bacteria and are responsible for the pathological effects: such as leukopenia, pyrexia, shock, and death in typhoid fever; pyrexia and shock in brucellosis of man; and abortion in brucellosis of domestic animals (168). On the other hand, in other gram-negative infections, endotoxin does not seem to contribute significantly to host damage. In infections with *V. cholerae*, enteropathogenic *E. coli*, *Vibrio parahaemolyticus*, and *P. aeruginosa* (35, 87, 171 [p. 129]), damage is not due to the endotoxins they contain but to recently recognized extracellular toxins. Participation of endotoxins in host damage is more likely when bacterial invasion of blood and tissues is extensive, because cell lysis and liberation of endotoxin might occur more readily than on body surfaces. This deeper invasion occurs in typhoid fever and in brucellosis but not in cholera and *E. coli* infections. The lessons of the study of endotoxins should be remembered in assessing the possible role of other microbial surface components in host damage.

The classical work with *M. tuberculosis* in guinea pigs showed that immunopathological phenomena involving microbial products can be dangerous and even fatal for the host. Also, skin tests indicate that hypersensitive states occur in many bacterial, viral, fungal, and protozoal diseases. Thus, in many infectious diseases, nontoxic microbial products could cause harm by sensitizing the host and then evoking any of the four types of reactions described by Coombs and Gell (170, 171 [p. 157, 383]): type I, reagenic, antibody-mediated, anaphylactic reaction; type II, antibody and often complement-mediated, cytotoxin reaction against cell-bound antigens; type III, antibody-antigen complex, Arthus-type reactions; and type IV, direct action of immune cells without the effect of antibody (171 [p. 157, 383]). The microbial products could be surface components either released from living microorganisms or liberated by lysis. Virus diseases in particular might be expected to entail immunopathological damage because obligate intracellular parasitism increases the chance that cell-bound antigens will be formed and that autoimmune responses will follow. Hence, immunopathology could be an important method of damaging the host, but proving this in a particular disease is not easy. Mere demonstration by a diagnostic test in vitro or by skin tests that an infected host is immunologically sensitive to microbial products is not proof in itself. The main pathological effects of the disease must be simulated by hypersensitivity reactions evoked in a sensitized host by products of the appropriate microbe. The remarks already made regarding liberation of surface toxins apply equally well to products that may evoke immunopathological effects; they must be liberated in vivo to be important in disease.

Bacteria

The endotoxins of gram-negative pathogens are the most important surface toxins. The literature on their structure, serological behavior, and biological activity is so informative (1, 161 [p. 302–333], 171 [p. 113], 184, 188) that I intend to add only a few points about their relevance to host damage. First, as stated above, the extent to which they are released in vivo determines the degree to which they are responsible for host damage (184). Second, endotoxins can be released from the cells during active growth as well as by lysis (188). Third, they are not confined to pathogenic bacterial species (188). Fourth, although toxicity is due to the common lipid A moiety and toxic effects are similar, potency varies with source: for example, *Brucella* and *Yersinia* spp. have relatively nontoxic lipopolysaccharides (188). Finally, toxicity derives mainly from damage to host cell membranes and from "triggering" complement by the alternate pathway (184, 188).

Group A streptococci are so well endowed with extracellular toxins (71) that a contribution to host damage by surface components seems superfluous. Nevertheless, this seems to happen with some cell wall components, notably the M-protein, but also with mucoprotein acting alone or complexed with polysaccharide C (71). Not only do these components seem to be set free during infection to produce toxic effects on leukocytes, lungs, joints, heart, and kidney, but, being resistant to host enzymes, they persist in the tissues after death of the streptococci and may contribute to chronic tissue damage (71, 171 [p. 157]).

Some mycoplasmas cause harm by producing extracellular toxins and H_2O_2 (171 [p. 217]), but others cause injury by the close association of host cells with their membranes, some of which may be inherently toxic (19, 161 [p. 143], 192). For example, the damaging effect of *Mycoplasma gallisepticum, Mycoplasma pulmoniae,* and *M. arthriditis* may be related to an affinity of their membranes for neuraminic acid receptors on host tissues (161, 171 [p. 217]). A close association between host cells and *M. pneumoniae* also has toxic consequences (28, 86). Cell membrane fractions from *M. pneumoniae* were toxic to hamster tracheal cultures, in contrast to other cell fractions (65). Similar results were obtained with membrane fractions from *Mycoplasma fermentans* (66), and possibly the same situation occurs with *M. mycoides* (141).

In the relatively few cases where immunopathological mechanisms cause major injury in bacterial disease (171 [p. 151]), surface components seem to play a part. Unfortunately, these components have rarely been identified. The pathology of tuberculosis appears to be due to cell-mediated hypersensitivity (type IV reaction) to products of *M. tuberculosis,* particularly the cell wall waxes (171 [p. 157]). Similarly, the cardiac, rheumatoid, and nephritic sequellae to streptococcal disease have been attributed, in part, to immunopathological phenomena in which the M-protein and C polysaccharide cell wall antigens cross-react with human heart muscle, and for some strains a membrane-associated antigen cross-reacts with human kidney (71, 171 [p. 157]). Most of the harmful reactions are either type II, with antibodies against streptococcal antigens producing cytotoxic reactions against host cell-bound antigens in heart and kidney tissue, or type III immune complex phenomena occurring particularly in kidney and vascular tissue (71, 171 [p. 157]). Endotoxins seem to cause damage due to hypersensitivity in some cases (18). Immunopathology also appears to play a part in syphilis; phospholipids of *Treponema pallidum,* which may be surface components, are related to the cardiolipins of host mitochondria (171 [p. 157]). Hence, antibody to the treponemal phospholipids could produce antibody-mediated cytotoxic activity against host cells. Delayed hypersensitivity also occurs in syphilis, but we do not know whether surface components of *T. pallidum* are involved (171 [p. 157]). There is similar uncertainty about the location of antigens responsible for the immunopathological effects in leprosy and brucellosis. Delayed hypersensitivity occurs in mycoplasmal disease (26), and the close association of mycoplasmal membranes with host cells could change their surface antigens, leading to hypersensitivity and to autoimmune effects (171 [p. 157]). However, neither the importance of these phenomena in mycoplasmal disease nor their causation by membranes is established.

Viruses

Viruses can damage host cells directly: by depletion of cellular components essential for life through the demands of the replication process, by inducing the production of cytotoxins within the cells or on their surfaces, and by the toxic action of virion components themselves (11, 58, 143, 166, 171 [p. 359]). The last two processes, called "fusion from within" and "fusion from without" (143), are caused by viruses (herpesviruses, paramyxoviruses, and poxviruses) that damage by inducing cells to fuse into polycaryocytes or syncytia.

The first toxic surface component of viruses to be described was the capsid penton of the adenovirus. In vitro, it caused cell rounding and detachment from glass (166), but its relation to damage in infection is not known. Although the hexon of adenovirus is not noticeably cytotoxic, it inhibits macromolecular synthesis (166) and, as an aggregate with antibody, seems more toxic to cells than a penton aggregate (97). Virus capsid components have also been implicated in the inhibition of protein synthesis by poliovirus (148) and the cytotoxicity of reovirus (172).

The fusion of cells by inactivated Sendai virus, herpesvirus, and Newcastle disease virus (143, 166) appears to be due to cell envelope components. Thus, fusion of cells in monolayers was induced by fragments of the envelope of Sendai virus (84). Tokumanu (171) concluded that the fusion factor of herpesvirus was a lipoprotein associated with the viral envelope, because a lipolytic agent and proteases reduced activity of the intact virus; in addition, fusion-promoting particles, having the physicochemical properties of lipoproteins, could be separated from homogenates. Similar work by others indicated that the fusion factor was in the envelope of Newcastle disease virus but that the intact envelope was necessary to promote full fusion activity (143). Cell envelope fusion factors have not yet been separated and identified, and all envelope fragments containing them have less fusing activity than the intact viruses (143).

The surface components of Newcastle disease virus may affect cytopathogenicity by virtue of their role in the release of virus from cells. A suggestion that cytopathic effects of virulent strains might result when the rate of formation of virus products is greater than the rate of release (2) received support from the increase in cytopathogenicity when virus release was inhibited by plant lectins (144).

Immunopathological mechanisms are likely to be responsible for host damage in virus disease, and viral surface components could play a large part. First, viruses incorporate host cell components into their surface structures and, thus, antibodies or cell-mediated reactions evoked by the virus can react with both normal and infected host cells. Second, virus-coded components foreign to the host are found in the membranes of the host cells; antibodies and cellular reactions to these changed cell membranes could harm both infected and normal host cells. The subject is too well documented to need further description (16, 22, 47, 58, 166, 171 [p. 383]). Potentially, any of the four types of immunopathological reactions could occur, and one or more of them do occur in two animal diseases – lymphochoriomeningitis in mice and Aleutian disease of mink (47, 171 [p. 383]). To what extent immunopathology is important in human virus disease is not clear.

Fungi

The classical fungal toxins such as aflatoxin are extracellular products formed in foodstuffs, and their role in infection is not clear (127, 171 [p. 1, 251]). Recently, toxins from the cell wall have been described which, if they are released, may be important in disease. A nephrotoxin, possibly of cell wall origin, was extracted from the washed mycelium of *Mortierrella wolfii*; when partially purified, it contained protein and lipid, and the protein was thought to be the toxic moiety. The cell walls of a virulent strain of *B. dermatitides* produced granulomatous reactions in mice similar to those seen in the human disease, and the mice died; in contrast, cell walls from an avirulent strain produced no granulomatous reaction although the mice died (34). Toxic preparations have also been obtained from *C. albicans*, and some seem to orginate in the cell wall (38). Hypersensitivity occurs in many mycoses, and immunopathological phenomena probably explain the pathology of some fungal skin diseases and respiratory mycoses such as farmer's lung (171 [p. 1, 251]). The products responsible are ill-defined, but they may come from the cell wall.

Protozoa

Neither extracellular nor cellular toxins have been isolated whose biological activity would explain the pathology of a protozoal disease (171 [p. 1, 269]). However, penetration of host cells seems to involve surface components: those at the conical ends of *Plasmodium* merezoites promote entry into erythrocytes (171 [p. 1, 269]), and the penetration factor of *T. gondii* is probably a membrane product (134). Immunopathological phenomena may contribute to the pathology of protozoal disease (171 [p. 1, 269]) and could involve surface components. For example, antibody-complexed surface glycoproteins are shed from trypanosomes in chronic disease and may be deposited in the kidney and other organs to produce Arthus-type reactions (37).

SURFACE COMPONENTS OF MICROORGANISMS THAT CONTRIBUTE TO HOST AND TISSUE SPECIFICITY

Two of the most striking and largely unexplored phenomena in microbial pathogenicity are host specificity – the ability of microbes to attack some animal species in preference to others – and tissue specificity, the ability of microbes to attack some tissues in preference to others (166, 169, 171 [p. 193, 415], 193). These specificities are determined by variations between species or tissues of the environment the host provides in relation to microbial proliferation. The most important host influences are: the nutritional environment ("replication factors" for viruses), the nature and strength of the humoral and cellular defense mechanisms, and, in relation to the route of entry of the pathogen, the host receptors for initial attachment and the barriers to dissemination. In some bacterial and viral infections, these host determinants of specificities have been identified (166, 169, 171 [p. 193, 415]). However, as expressed in the introduction, a description of such host influences is excluded from this review. On the other hand, microbial factors that are complemented by the environment of the susceptible host and are unaffected or antagonized in the resistant host are subjects for discussion.

Bacteria

The most prominent role that bacterial surface components can play in host and tissue specificity is in mediating selective adherence to the mucous surface at the initiation of infection (68, 70). Examples of selective adherence to tissues are as follows. Pathogenic *S. pyogenes* attached to human pharyngeal cells better than *E. coli*, correlating with the fact that the former and not the latter infects the oral cavity (51, 69). *V. cholerae* and *E. coli* attached preferentially to the epithelium of the upper rather than the lower bowel, whereas *S. flexneri* adhered to colonic cells rather than to those of the upper bowel (171 [p. 25]). With regard to host specificity, *S. pyogenes*, which rarely infects rodents, attached more strongly to human than to rodent buccal epithelial cells (70). Also, *S. salivarius* and *S. sanguis*, which are found naturally in humans but not in rats, adhered better to human tongue cells and less well to rat tongue cells than *S. faecalis* and a serum-requiring diphtheroid, which are found naturally in rats (68).

Unfortunately, in most cases neither the bacterial surface components nor the complementary host receptors on susceptible tissue are known, although the M-protein of *S. pyogenes* and the K88 antigen of some enteropathogenic *E. coli* seem to determine adherence to the buccal cavity and upper small intestine, respectively (51, 80).

Viruses

Evidence for the influence of virus surface components on host and tissue specificity has been obtained for the following four stages of replication: attachment and penetration, uncoating, assembly, and release. A combination of work in animals and in primary cell culture was used to elucidate the examples of the first two stages, but only cell cultures or eggs were used for other examples.

Adsorption and penetration. The importance to susceptibility of interaction between virus surface components and host cell receptors was shown by Holland and his colleagues (81), with poliovirus and primate and nonprimate tissue. Cultured human and monkey cells susceptible to poliovirus infection adsorbed virus with first-order reaction kinetics. In contrast, nonprimate cells insusceptible to poliovirus infection did not adsorb virus. When susceptible cells with adsorbed virus were disrupted, host cell membrane fractions had associated virus, whereas insusceptible cells treated with virus yielded membrane fragments without associated virus. In humans and rhesus monkeys, poliovirus infects the intestine and the central nervous system but not other tissues. Correspondingly, homogenates of human and monkey intestine, brain, and spinal cord adsorbed poliovirus strongly, whereas homogenates of other tissues did not, with the unexplained exception of liver. The importance of envelope-cell receptor interaction in determining host and tissue specificity was underlined by experiments in vitro and in vivo, showing that cells insusceptible to infection with intact virus translated envelope-free poliovirus RNA if this was introduced into the cell. First, with the RNA itself, one cycle of poliovirus replication was produced, not only in cultures of rodent and avian cells, but also in mice after intracerebral inoculation. Second, poliovirus genomes were enclosed within the capsids of a pathogenic mouse enterovirus, coxsackievirus B_1. This "mixed" virus produced one cycle of poliovirus replication in cell cultures and in mice after intracerebral inoculation (32). The infection was stopped by antiserum to coxsackievirus B_1, presumably by complexing with the coxsackievirus B_1 capsids, thus preventing them from interacting with the mouse cell receptors and so promoting virus entry. In both experiments, only one cycle of replication occurred because the released poliovirus possessed the "wrong" capsids for further cell entry. This work was completed over 10 years ago. Unfortunately, there has been little progress since. Neither the nature of the operative group on the virus surface nor the host cell receptors are known, but protein fractions from membranes of susceptible cells, in contrast to those of insusceptible cells, interacted with the envelope of poliovirus, leading to uncoating and release of nucleoprotein (24).

The avian RNA tumor viruses provide another example of host specificity determined by the interaction between virus surface components and host receptor substances (156, 187). Some chicken lines are resistant to infection with some strains of tumor virus, and their cells fail to adsorb the viruses in whole-animal or in cell culture experiments. The experiments were similar to those with poliovirus. The leukoviruses have been grouped into five antigenic types (A, B, C, D, and E) by adsorption studies with various lines of chicken cells. The cell receptor substances that interact with the virus envelope proteins seem to depend for their production on so-called tv (tumor virus) genes a, b, c, d, and e. The natures of the virus envelope components and the chicken cell receptors are not known. However, mere attachment of virus to the cell is insufficient for infection to take place, since virus particles adsorb to both susceptible and resistant cells but penetrate only susceptible cells (36, 142). A different type of binding may occur between cell surface and virus envelope at the fully compatible sites that allow penetration (12). Cellular resistance to a virus type can be overcome, as for poliovirus, by phenotypically mixing its envelope proteins with envelope proteins from a virus type that infects the cell (77, 187). Host cell resistance has also been overcome by the intracellular inoculation of virions using Sendai virus fusion between resistant cells and cells containing the virions (191).

Type A feline leukemia viruses infect only feline cells, whereas type B viruses can also infect human and dog cells. Experiments with phenotypically mixed virus have shown that complementarity between the virus envelope and host cell receptors is responsible for the observed patterns of cellular specificity (90). As for poliovirus and the chicken RNA tumor viruses, the nature of the cell surface receptor is not known.

Successful interaction between virus surface components and host cell receptors does not always determine host or tissue specificity. Sometimes the receptors for the viral component are ubiquitous, as are those for the myxo- and paramyxoviruses. For example, in organ culture, influenza virus adsorbed equally well to susceptible (respiratory and urogenital tissues) and insusceptible ferret tissues (182). In these cases, susceptibility or resistance is determined at a later stage in replication. Thus, for influenza virus, the cleavage of the surface hemagglutinin is essential for infectivity, and its occurrence seems to depend on the host cell (100); similar remarks apply to cleavage of the glycoprotein of Newcastle disease virus (129).

Uncoating. The HMV (PRI) strain of mouse hepatitis virus infected PRI but not C_3H mice. The host specificity was reflected in the differential ability of the virus to infect and destroy liver and peritoneal macrophages from these two types of mice (166, 169, 171 [p. 415]). In kinetic experiments, virus adsorbed to and penetrated both resistant and susceptible macrophages. However, after adsorption and penetration, eclipse and replication ensued only in the susceptible cells; infectious virus persisted for a few days in the insusceptible cells and then declined. Thus, susceptibility and resistance of the mice to hepatitis virus appears to be determined by the presence or absence of a system in the liver macrophages which is involved in removing the virus surface coat.

Assembly. Sendai virus passaged in embryonated eggs infected both eggs and a variety of mammalian cells. However, after one growth cycle in mammalian cells, the progeny virus remained infectious for eggs, but not for mammalian cells. When repassaged in eggs, the progeny virus regained its capacity to infect mammalian cells (160). Differences in the viral envelope glycoproteins of Sendai virions grown in eggs and in mammalian cells were identified (83, 160). The low infectivity of Sendai virus grown in mammalian cells was attributed to lack of post-transitional cleavage of one of the two virus envelope glycoproteins during the final stages of virus assembly and release at the plasma membrane (83, 160). In the egg, cleavage occurred normally. Cleavage of the intact envelope glycoprotein of virus grown in mammalian cells can be achieved by trypsinization of isolated virions, and this confers ability to infect further mammalian cells. Cleavage of the viral envelope glycoproteins in the egg, which occurs at cell surfaces, is believed to result from the action of host cell proteases acting directly or indirectly by activating proteases in body fluids and serum (73).

Release. Simian virus 5 budded freely from the plasma membrane of monkey kidney cells, whereas in hamster cells much smaller amounts of infective virus were released, despite intracellular accumulation of viral subunits (99). Also, for paramyxo- and myxoviruses, the synthesis of the viral M protein, which can vary significantly in different cell types, seems to be a critical factor in controlling the rate of release of these viruses at the cell surface. It may thus account for observed differences between cells in ease of virus release (29, 154). A similar situation, in which differences in cellular susceptibilities are reflected in variations in rate of release of virus at the cell surface, occurs for Newcastle disease virus (2).

Fungi and Protozoa

Host and tissue specificities occur in fungal and protozoal infections but, as far as I know, any surface components concerned have not been identified.

CONCLUSIONS

This survey reaches four conclusions on the influence of microbial surfaces on pathogenicity.

(i) Surface components are often major determinants of pathogenicity, affecting all stages and aspects of disease production.

(ii) Although the involvement of microbial surfaces in pathogenicity is certain, the chemistry of the components concerned is known only in a few cases, as in the bacterial determinants of adherence to mucous surfaces and those responsible for resistance to ingestion by phagocytes. However, these examples show that, with sufficient effort, surface virulence determinants can be identified.

(iii) Surface components of pathogens change in the environment in vivo, due to phenotypic influences and selection, and these changes in surface components may be important in pathogenicity.

(iv) In most cases the pattern for future research is indicated by the studies of bacteria, since more knowledge has accumulated about them than about other microbes. But sometimes work on microorganisms other than bacteria has pointed the way forward, for example, in the antigenic shift of the trypanosomes and in the explanations for host and tissue specificity of viruses.

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[1] To avoid an excessive list of references, many points have been referred to six key reviews from which the original references can be obtained: 140, 161, 166, 170 ,171, and 186.

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